SCIENTIFIC ABSTRACT

Cell death is an essential event in the formation of any multicellular organism. From early stages of the organism's embryogenesis large number of cells die daily.

Several types of cell death are known today: violent death induced by unfavorable treatments, cell death induced by cytotoxicity, normal aging, pathological cell death and programmed cell death.

"Programs or naturally occurring cell death" is of a great importance for normal development. It involves much of the body sculpting by removal of unneeded or obsolete cells. During embryo genesis neuronal cells death appears to adjust the number of cells to the size of the target they inervate.

What is the mechanism underlying the cascade of events leading to naturally occurring cell death? Cell death could thought to be a passive process resulting from the loss of cellular component. But this does not seem to be the case . Programmed cell death appears instead to be an active process which requires transcription and translation, which lead to the suggestion that a "cell death program" exist normally that it triggered by expression of specific genes and proteins. At present little is known about the death program and even less is known about the genes, the proteins and their functions.

In the embryo 50% of the sympathetic sensory or motor neurons die due to deprivation of trophic factors that are available in limited amounts. Since Nerve Growth Factor (NGF) is the most thoroughly studied, physiologically proven and generally available neurotrophic factor, the study of the mechanisms that activate neurotrophic factor is focused in most cases to the study of NGF. The current hypothesis is that neurons (particularly immature neurons) are programmed to die and possess an endogenous "suicide program" for this purpose. The current thinking is, that since neuronal death is an active process NGF acts to suppress this program. Thus, the survival promoting effects of NGF would result from an ability to suppress death rather than an ability to nourish life.

Support to this notion that the "death program" involved an activation of a cascade of new mRNA and protein synthesis comes from the work of few laboratories that studied the effect of macromolecular synthesis (RNA and protein) on neuronal death due to NGF deprivation. They have found that neurons that have been deprived of neurotrophic factors and usually die, survived instead in the presence of macromoleclar synthesis inhibitors. They suggested that the inhibitors of RNA and protein synthesis non-selectively mimic the ability of NGF to suppress the expression of these genes and proteins that induced cell death.

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Research goals: The long-term goal of this research is to develop molecular markers of neurodegenerative processes. The approach toward this goal will be to study the basic cellular mechanism underlying neuronal death. In this pursuit, I hope to understand the mechanism by which neurons die in natural and in neurodegenerative disorders. The long term objectives of this project will be pursuit by experiments directed at the following specific goals:

1.To identify and characterize the proteins and genes that activate the death program.

2.To purify these proteins, to obtain partial amino acid sequence and to clone the corresponded cDNA.

3.To assess the function of the death associated proteins by employing "knock out" experiments. That will be done by using nucleic acids probes (antisense)to block the translation of these proteins and or antibody probes to block the function of these molecules.

4.To determine whether or not the death program is a general program which activates cell death in other cell types including neurodegenerative disorders.

5.To use immunocytochemistry and in situ hybridization to characterize the distribution of death associated proteins in vitro and in vivo with particular emphasis on situation of normal and experimentally induced cell death in the nervous system

Research plan and methods: To explore the death phenomenon a broad range of scientific disciplines and techniques will be employed

To study the biochemical mechanism of programmed neuronal cell death, I will utilize a model system which consists of a primary culture of homogenous population of dissociated sympathetic neurons isolated from the superior cervical ganglion of newborn rats. These cells depened on NGF for their survival and removal of NGF is the trigger to activate the death program.

Identification and characterization of death associated proteins will be done by using the analytical power of two-dimensional gel electrophoresis. Recent works have identified about ten proteins that alter their synthesis due to neurotrophic factor deprivation. None of these proteins has been characterized yet. The proteins that will show alterations in their synthesis due to NGF deprivation will be linked to the death process by using pharmacological agents that prevent cell death ($K^+$, elevated $[Ca^{++}]_{in}$, and cAMP). Proteins that will be affected by these pharmacological agents will be categorized as associated to the death process.

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The identified death associated proteins will be purified using preparative 2-D gels. To obtain partial amino-acids sequence the purified protein will be first subjected to partial proteolysis to overcome the problem of blocked amino terminus and than sequenced using Edman degradation. To clone the corresponding cDNA, libraries will be screened using degenerate oligonucleotide synthesized according to the partial amino-acid sequence previously obtained, or if more than one partial amino acid sequence is available polymerase chain reaction will be employed. To assess the biological role of these proteins we will use "knock out" experiments. Blocking the translation of the death associated proteins and subsequently Their function, that will be done by intracellular injection of antisense oligonucleotides. Similarly, the function of these proteins will be blocked by microinjection of antibodies. The proteins and oligonucleotides probes which generated in the study of NGF deprived cell death would be employed to test the idea if genes and proteins which activate the death program of NGF deprivation paradigm are common to those that play role in program cell death of other neuronal and non-neuronal preparations. In particular we plan to apply this approach to investigate programmed neuronal cell death during development and and non-neuronal programmed cell death such as glucocorticoides treated thymocytes or IL-2 deprived T cells. In addition to these basic neurobiological issues the possible role of the program cell death machinery in neurodegenerative diseases such as Alzheimer and Parkinson will be explored.

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